Biochemical and neurophysiological parameters of acute brain injury

Elting, Jan Willem J.

Chapter 3

The AMPA antagonist ZK200775 in patients with acute ischemic stroke: Possible glial cell toxicity detected by monitoring of S-100B serum levels

Jan Willem Elting, Geert Sulter, Markku Kaste, Kennedy Lees, Hans Diener, Marc Hommel, Mark Versavel, Albert Teelken, Jacques De Keyser

Stroke 2002;33:2813-2818

Abstract

Objective: S-100B and neuron specific enolase (NSE) serum concentrations can be used as peripheral markers of glial cell and neuronal damage, respectively. We investigated these markers in a clinical trial with the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) antagonist ZK200775 in acute ischemic stroke patients.

Methods: In a multicenter double blind, randomized, placebo-controlled phase II trial, 61 ischemic stroke patients were treated with either placebo or active drug in a dose finding design. Twenty-five patients received placebo, 12 patients received a total dose of 262,5 mg in 48 hours (dose group 1) and 13 patients received a total dose of 525 mg in 48 hours (dose group 2). Eleven patients received a total dose of 105 mg over a period of 6 hours (dose group 3: reduction of total dose and infusion time due to adverse events in group 2). Serum concentrations of S-100B and NSE were analyzed using a monoclonal sandwich immunoluminometric assay. Neurological outcome was assessed using the National Institutes of Health Stroke Scale (NIHSS).

Results: In group 2 there was a significant transient worsening in the mean NIHSS score 48 hours after the start of treatment. The mean increase was 11 points. This was due to reduction of consciousness (stupor and coma) in 8 out of 13 patients. Neurological deterioration in group 2 was associated with a higher increase of S-100B concentrations, but not of NSE concentrations, than in the placebo group. The trial was stopped prematurely for safety reasons.

Conclusions: The AMPA antagonist ZK200775 transiently worsened the neurological condition in patients with acute ischemic stroke. Our results suggest that besides neuronal dysfunction, glial cell toxicity may have occurred. It may be useful to introduce monitoring of serum markers of brain damage in phase II trials with glutamate receptor antagonists.

Introduction

Neuroprotective drugs are effective in animal stroke models, but have failed to show clinical efficacy in stroke patients [1-3]. In the ischemic penumbra there is a build-up of extracellular glutamate, which leads to an overstimulation of N-methyl-D-aspartate- (NMDA) and AMPA-type glutamate receptors. This is associated with excitotoxicity due to an accumulation of calcium in the cells. Among the glutamate receptor blockers, NMDA antagonists have been the most frequently studied, but side effects is one of the factors limiting their use in humans [4]. AMPA antagonists also hold promise as neuroprotective compounds in acute ischemic stroke, but so far they have not reached phase III clinical trials [5]. ZK200775 is a quinoxalinedione-like drug with high affinity at the AMPA/kainate receptor site with little activity at the NMDA site. It behaves as a neuroprotective drug in rodent models of ischemia and head trauma [6]. Three phase I studies in healthy subjects showed acceptable side effects at dosages required to obtain serum levels that were neuroprotective in the animal models. Side effects included mild and reversible sedation, visual disturbances and memory impairments (Unpublished Schering Research Reports AG99, AO12 and AO13).

S-100B and neuron specific enolase (NSE) can serve as peripheral markers of brain damage in various neurological diseases [7, 8]. S-100B exists predominantly in glial cells, while NSE is mainly found in neurons. In patients with acute ischemic stroke, serum levels of these proteins correlate with infarct-size, as well as with neurological and functional outcome [9-11]. Temporal patterns of both proteins are usually of the biphasic type with peak levels around the 2nd to 4th day after stroke onset [12, 13]. It has been suggested that these biochemical serum markers could serve as surrogate tools to evaluate neuroprotective therapies [1, 9, 10, 14].

The AMPA antagonist ZK200775 was studied in a phase II multicenter, double blind, randomized, placebo-controlled dose-escalating trial, to evaluate safety in patients with acute ischemic stroke. The details of the clinical and pharmacokinetic data will be reported elsewhere, but will be briefly mentioned here. In this article we assessed if there were any treatment effects on the serum concentrations of S-100B and NSE. Efficacy was not a primary goal of this study, but the National Institutes of Health Stroke Scale (NIHSS) was used as a secondary outcome parameter.

Methods

Patients

Patients were included from august 1997 to august 1998 in 10 hospitals across Europe (see Appendix). Ethics Committee approval was obtained at each study center. Inclusion criteria were a stroke severity on the NIHSS between 2 and 21. For patients who developed symptoms overnight, stroke onset was defined as the last time the patient was known to be symptom free. Female patients were included only if they were post-menopausal or surgically sterilized. Exclusion criteria included cerebral hemorrhage, renal insufficiency, ventricular arrhythmia (atrial fibrillation was not an exclusion criterion), use of thrombolytics, severe hypertension and severe concomitant disease likely to influence clinical assessment during the study (such as dementia or metastasized cancer). Other drug treatments such as aspirin and heparin were allowed.

All patients gave their written informed consent to participate in this study. Written consent was also accepted from the next of kin or close family member if the patient was unable to write. Intact ability to understand information and to communicate (best NIHSS language score 0 or 1, best NIHSS dysarthria score 0 or 1) was an additional requirement for inclusion.

Treatment was started within 24 hours of stroke onset in all patients. Patients were randomly assigned to either placebo or trial drug. The study was directed by a steering committee and safety was monitored by an independent safety board (see Appendix). An interim safety analysis was performed after each dose group, after which decisions were made to proceed with a higher dose or adjusted dosing. The first dose group received a loading dose of 25 mg in 30 minutes followed by a maintenance dose of 237,5 mg over 47 hours and 30 minutes. The second group received a loading dose of 50 mg in 30 minutes followed by a maintenance dose of 475 mg over 47 hours and 30 minutes. The third group received a loading dose of 50 mg in 30 minutes followed by a maintenance dose of 55 mg over 5 hours and 30 minutes. This reduction of total dose and infusion time was decided after the end of dose step 2 due to adverse events in group 2. This study was part of a first phase II study that focused on safety and tolerability. Sample size was therefore determined on a pragmatic basis.

S-100B and NSE

Venous blood samples were taken on admission (baseline), after 24 hours, 48 hours, 72 hours, 96 hours and after 7 days. Within 1 hour of collection, all samples were centrifuged and stored at -20°C until analysis. After clotting and centrifugation at 4000 rpm for 10 min, NSE and S-100B were analyzed with the use of monoclonal sandwich immunoluminometric assays (Sangtec) and a fully automated LIA-mat system.

Hemolysis has no influence on serum S-100B levels but can increase serum NSE levels [15,16]. Therefore, hemolytic samples were identified by visual inspection of the serum and the amount of NSE attributable to hemolysis was determined by using the following formula : NSE $=0{,}665 \times$ hemoglobin $- 0{,}533$. This equation was obtained from earlier experiments in which it was shown that increasing levels of free serum hemoglobin correlated highly significantly ($r=0{,}998$) with serum NSE levels (A. Klaren; unpublished data, 1998). This amount was subtracted from the total amount of serum NSE. Samples in which the estimated amount of NSE as a result of hemolysis was > 50% were left out of the analysis.

Neurological assessment

Stroke severity was assessed with the NIHSS on admission, after 2 days, after 7 days, and after 4 weeks. Efficacy analysis was not a primary goal of this study because of safety aspects and a wide admission window of 24 hours, but the difference in NIH stroke scale score between week 4 and admission was used as a secondary "outcome parameter". Patients were considered to have a neurological improvement when the difference between these scores was ≥ 4 points, no major change when the difference ranged from 3 to –3, and neurological deterioration when the difference was ≤ -4 points [17].

Statistics

To analyze S-100B and NSE data, the area under the curve (AUC) and the individual peak levels were used as summary measures [18]. Peak values were not determined if there was a missing value directly before or after the peak level, unless the missing value was on day 7, since earlier studies showed that this is an unlikely time point for peak levels of both serum markers. AUC values were not calculated if there was more then 1 missing value, or if the first or last value was missing. Interpolation was used if missing values on other time points were present. Log transformation was used when these variables followed a non-normal distribution. One-way analysis of variance (one-way ANOVA) was performed to compare the S-100B and NSE data between treatment groups. Correction for multiple comparisons was performed using the Dunnett T3 method for unequal variances or the Bonferroni method in case of equal variances [19]. To compare proportions between groups, χ^2 tests and Fisher exact tests were used. Multiple regression analysis with a backward stepwise strategy (probability of F for removal ≥ 0,1, probability of F for entry ≤ 0,05) was used to assess the influence of several baseline variables on maximum S-100B values.

Results

Stroke type and severity

Demographic and baseline characteristics of the placebo group and the 3 active treatment groups (termed group 1, group 2, and group 3) are shown in Table 1**.** With regard to baseline characteristics group 1 had a higher percentage of stroke due to small vessel disease (50%) than the other groups. In group 1 hypertension and diabetes were present more often and in group 2 there were more previous non-disabling strokes or TIA’s than in the placebo group.

Characteristics	Placebo group	Dose Group 1	Dose Group 2	Dose Group 3
Total dose		265,5 mg/48 h	525 mg/48 h	105 mg/6 h
Time between stroke onset and start trial drug (hours)	16,3 ± 5,2	15,7 ± 4,6	17,3 ± 6,7	18,2 ± 4,0
% SAE resulting in discontinuation of trial drug	4	0	69	9
Time between start trial drug and discontinuation (hours)	23	NA	21 ± 14	23
Number of patients	25	12	13	11
Sex (M/F)	12/13	10/2	8/5	9/2
Age (mean ± SD)	67,5 ± 11,4	65,9 ± 8,8	69,7 ± 8,8	63,1 ± 13,2
Weight (kg)	75 ± 17	83 ± 13	75 ± 16	81 ± 17
Large vessel occlusion Vs lacunar infarction (%)	72 vs 28	50 vs 50	92 vs 8	91 vs 9
History of (%)				
Cardiac disease	48	50	31	36
Hypertension	32	67*	54	45
Diabetes	4	25*	15	0
Hypercholestrolaemia	28	17	8	18
Previous Stroke or TIA	8	25	38*	18

Table 1. Demographic and clinical data. * $p<0,05$ vs placebo. Note the large percentage of lacunar strokes, hypertension and diabetes in group 1. SAE indicates Serious Adverse Event; NA, not applicable.

Baseline NIHSS scores were similar for the 58 patients used in the S-100B and NSE analysis ($F=1,8$ $p=0,16$) with comparable baseline values between the placebo group (6,3 ± 4,4), group 3 (7,0 ± 4,1) and group 2 (7,5 ± 4,4), but lower baseline values in group 1 (4,0 ± 2,2).
After 48 hours a highly significant increase was seen in NIH stroke scale scores in group 2 ($p=0,008$ compared to placebo, $p=0,018$ compared to group 3, and $p=0,03$ compared to group 1). Eight out of 13 patients in group 2 had a reduction of consciousness, 1 had a confusional state, and one showed stroke progression. The maximum incidence and intensity of these adverse events usually occurred > 24 hours after the start of infusion (range 4-48 hours). These events led to a prompt discontinuation of study medication. In the placebo group and group 3, there were 1 and 2 patients, respectively, with events of comparable severity, but mild somnolence was more frequently observed. One patient died after 96 hours in group 2 because of cerebral edema with transtentorial herniation, and 2 patients died of severe pulmonary edema and progressive stroke after 4 weeks in the placebo group. Monitoring of

vital parameters (blood pressure, pulse, O2 saturation, temperature) and blood glucose showed no major differences between the groups.

The NIHSS score at 4 weeks was highest in treatment group 2, but did not differ significantly from the other groups (F=2,423 p=0,07). When categorized in 3 outcome groups reflecting deterioration, no change or improvement, there was no difference in outcome between treatment groups (Fishers exact test: group 3, vs placebo p=0,81, group 1 vs placebo p=0,08, group 2 vs placebo p=0,70). Figure 1 shows the NIHSS scores in the placebo group and the 3 active treatment groups.

CT scanning was performed in all patients at baseline and in 77% of patients at day 7, depending on the judgement of the investigator. Baseline CT scan results were consistent with ischemic stroke in all patients. Two patients had hemorrhagic transformation of the infarct on day 7. The trial was stopped prematurely because of safety concerns, especially the reduction in consciousness.

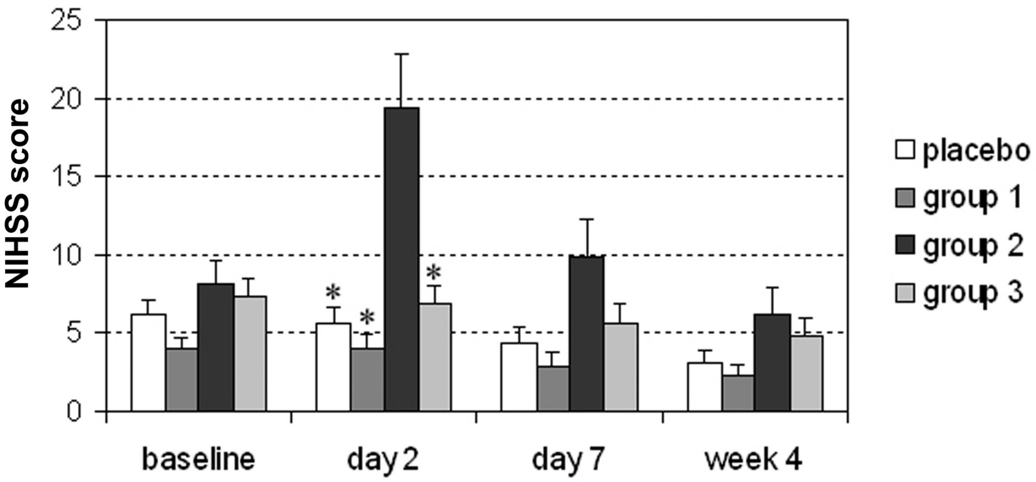

Figure 1. Mean ± SEM of NIHSS scores at all time points for all treatment groups (n=58). * p<0,05 vs group 2 (ANOVA, Bonferroni adjustment). Deceased patients and patients with hemorrhagic transformation of the infarct were excluded (n=3). Group 2 received the highest dose of ZK200775, and group 3 the lowest dose. The increase in mean NIHSS score in dose group 2 was due to reduction of consciousness (stupor and coma) which was transient in nature. After 4 weeks no differences in NIHSS scores were found between the groups.

S-100B and NSE

S-100B values were obtained for 96% of all intended samples; 4% were lost because of non-sampling or death of the patient. NSE values were obtained for 94 % of all intended samples; 5 % were lost because of non-sampling or death, and 1 % were lost because of uncorrectable amounts of hemolysis. Nine percent of all NSE samples were hemolytic but could be corrected using the correction formula. The patient in group 2 who died after 96 hours was excluded from the analysis because neither peak levels nor AUC could be calculated reliably. The maximum serum levels of both markers were very high in this patient (S-100: 11,25 ng/ml, NSE: 22,18 ng/ml) but were obtained during the stage of transtentorial herniation, and were therefore considered unreliable. Two patients with hemorrhagic transformation of the infarct were excluded from the analysis because cerebral hemorrhage causes different S-100B and NSE patterns [20]. The remaining 58 patients entered the S-100B and NSE analysis.

The evolution over time of the S-100B and NSE levels in all treatment groups between admission and day 7 is shown in figures 2 and 3, respectively.

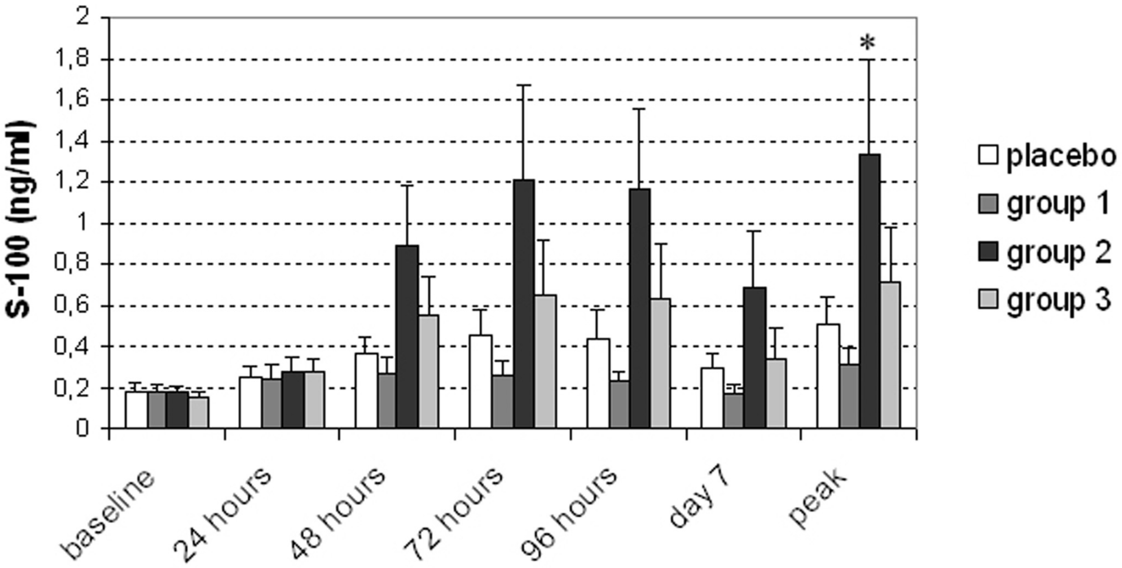

Figure 2. Mean ± SEM of S-100B on all time points for all treatment groups. Peak represents mean of individual peak levels and was used as a summary statistic for statistical testing. * p<0,05 vs placebo (after log transformation; ANOVA, Bonferroni adjustment).

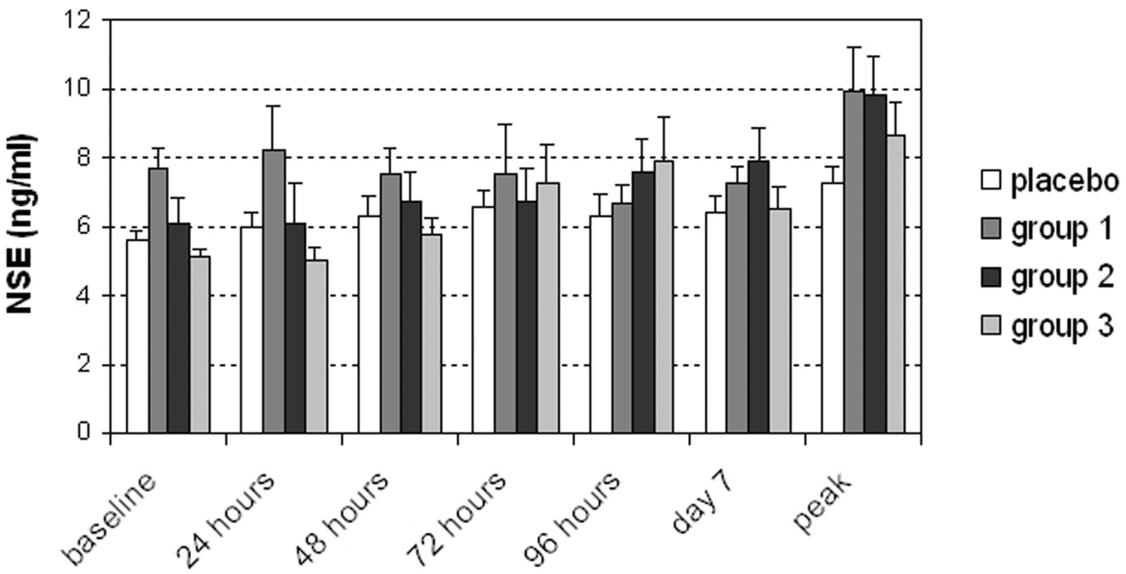

Figure 3. Mean ± SEM of NSE on all time points for all treatment groups. Peak represents mean of individual peak levels. No significant differences were found.

Individual peak levels of S-100B could be determined in all but 1 patient and for NSE in all but 5 patients (all because of missing values). In 4 patients AUC values for S-100B and in 13 patients AUC values for NSE could not be determined because of missing values at baseline or at 7 days.

NSE values followed a normal distribution after corrections for hemolysis were done. Individual maximum values and AUC did not differ significantly between the treatment groups. S-100B values followed a skewed distribution in all groups, which was normalized after log-transformation. Compared with the other groups, S-100B levels in group 2 were higher between 48 and 96 hours after start of treatment. There was a significant difference in individual maximum values between group 2 and the placebo group (F=3,46 p=0,023; post-hoc p=0,047). AUC analysis showed no significant differences between the groups (F=1,244 p=0,303). To investigate possible influences of differences in baseline characteristics on S-100 results, we performed a multiple regression analysis using the NIHSS baseline value, stroke type (lacunar versus large vessel occlusion), time between stroke onset and start of treatment, and the treatment group variable (ordered according to increasing dose, 0=placebo 3=highest dose) as independent variables. In the final model, baseline NIHSS score and treatment group were included as independent variables, and time of treatment and stroke type were excluded (table 2).

Independent Variable	P-value
Final model	
NIHSS baseline	< 0,001
Group variable	0,016
	Adjusted R^2 0,41
Excluded Variables	
Stroke Type	0,29
Time stroke onset-treatment	0,74

Table 2. Results of a backward stepwise multiple regression analysis with S-100B maximum levels as the dependent variable. In the final model only NIHSS score at baseline and the treatment group variable (ordered according to increasing dose) contributed significantly.

Discussion

Several AMPA antagonists have been developed as neuroprotective compounds and entered clinical development. None of these compounds has reached phase III clinical trials, and unacceptable adverse events can be the main obstacle. The most prominent finding of this study was that the administration of the AMPA antagonist ZK200775 in ischemic stroke patients resulted in a transient neurological deterioration, which was associated with a higher than expected rise in serum S-100B levels. The level of sedation was more severe in stroke patients and occurred later than in normal subjects during the phase I studies. Besides a longer infusion time and higher doses in stroke patients, blood-brain barrier disruption, with increased tissue concentrations may be responsible for these differences. Although baseline stroke characteristics were not equally distributed in all treatment groups, this is not a sufficient explanation for the difference in serum S-100B levels between the placebo group and dose group 2. In fact, the placebo group, group 2 and group 3 were quite similar, but group 1 consisted of less severe strokes. There was a relation between S-100B levels and stroke severity, but the results of the multiple regression analysis indicate that ZK200775 treatment had an independent effect on S-100B levels. In the present study, time to drug administration was relatively late for a putative neuroprotective compound, and we cannot rule out that the results may have been different when the drug had been given within 3-6 hours after stroke onset. However, the available data show no significant influence of time to treatment on the S-100B levels. The drug did not affect serum NSE levels.

It is tempting to hypothesize that the drug caused a reversible neuronal dysfunction while having a toxic effect on glial cells. This is different from neurotoxicity, which would rather lead to an increase in serum NSE levels, as we have previously shown in patients with hyperglycemic cortical ischemic stroke [21]. Direct suppression of synaptic transmissions via neuronal AMPA receptor blockade can explain reduced neuronal activity, as some in vitro experiments suggest [6]. This has also been demonstrated in animal experiments with AMPA antagonists; local glucose metabolism decreases in rats treated with 2,3- dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline (NBQX), a selective AMPA antagonist with a biochemical and pharmacological profile similar to that of ZK200775 [22,23]. This reflects decreased neuronal metabolic demands during decreased synaptic transmission. Multiple cortical areas but also deeper brain structures such as the thalamus were involved. These phenomena were accompanied by side effects similar to those observed in this study. Moreover, brain stem structures may also have been involved since subcortical EEG signs were found in healthy subjects receiving ZK200775 (Subcortical and cortical signs detected visually in pharmacological electroencephalograms of healthy male volunteers are induced by the competitive AMPA antagonist ZK200775; H. Ott and W. Scheuler, unpublished data, 2000).

Glial cell damage can also contribute to neuronal dysfunction since they are very important for neuronal homeostasis; there are several known mechanisms by which astroglial damage leads to neuronal dysfunction [24]. One of these is interference with neuronal glucose metabolism in which astrocytes play an important role [25]. We can only speculate about the mechanism by which ZK200775 may have caused glial cell toxicity. While nothing is known about a possible toxic effect of AMPA antagonists on astrocytes, a direct effect cannot be excluded. Presynaptic AMPA receptors, which regulate the synaptic release of glutamate, exist [26,27]. Blockade of these receptors by AMPA antagonists can lead to a decrease in extracellular glutamate levels [28]. Since glutamate is an essential element for glutathione synthesis in astroglia, the consequence is a lowering of the astrocyte antioxidant defense system, leading to an increased vulnerability to free radical damage [29,30]. Support for this theory is that inhibition of the glutamate transporter by alpha-aminoadipic acid also leads to a decreased intracellular availability of glutamate and subsequent selective damage to astrocytes [30-32].

Our study is another example of how toxicity of glutamate antagonists may prohibit their clinical use. A trend towards increased mortality was observed in stroke patients treated with the NMDA antagonist Selfotel, and this was interpreted as a neurotoxic effect [33]. The present study suggests that AMPA receptor antagonists may have toxic effects on glial cells, at least in patients with brain ischemia. We suggest that monitoring of serum markers of brain damage should be included in phase II trials with glutamate receptor antagonists.

Appendix

Steering Committee

The following are in alphabetical order: *Dr H.C. Diener*, Abteilung Neurologie, Universität Essen, Germany. *Dr M. Hommel*, Service Neurologie, CHU Hopital Michalon, Grenoble, France. *Dr M. Kaste*, Department of Neurology, Helsinki University Central Hospital, Helsinki, Finland. *Dr J. De Keyser*, Academisch Ziekenhuis Groningen, Groningen, Netherlands. *Dr K.R. Lees*, University Department of Medicine & Therapeutics, Gardiner Institute, Western Infirmary, Glasgow, UK. *H. Steiner*, Schering AG, SBU Therapeutics CV/CNS, Berlin, Germany. *Dr M. Versavel*, University of Antwerp, at that time at Schering AG, SBU Therapeutics CV/CNS, Berlin, Germany.

Safety Board

The following are in alphabetical order: *Dr W. Hacke*, Department of Neurology, University of Heidelberg, Heidelberg, Germany. *Dr J. Mau*, Department of Statistics in Medicine, Heinrich-Heine Universität, Düsseldorf, Germany. *Dr K. Poeck*, Medizinische Fakultät der RWTH Aachen, Aachen, Germany.

Study Centers and Principal Investigators

The following are ordered according to decreasing numbers of stroke patients contributed to the study; see protocol dated February 5, 1999: *Dr M. Kaste*, Department of Neurology, Helsinki University Central Hospital, Helsinki, Finland. *Dr Busse*, Neurologische Klinik, Klinikum Minden, Minden, Germany. *Dr K.R. Lees*, University Department of Medicine & Therapeutics, Gardiner Institute, Western Infirmary, Glasgow, UK. *Dr Chr Hedman,* Päljät-Häme Central Hospital, Lahti, Finland. *Dr H.C. Diener*, Abteilung Neurologie, Universität Essen, Essen, Germany. *Dr P. Marx,* Freie Universität Berlin, Universitätsklinikum Benjamin Franklin, Berlin, Germany. *Dr J. De Keyser*, Academisch Ziekenhuis Groningen, Groningen, Netherlands. *Dr J. Sivenius*, Kuopio University Hospital, Kuopio, Finland. *Dr J. Caekebeke,* Onze Lieve Vrouw Ziekenhuis, Aalst, Belgium. *Dr M. Hommel*, Service Neurologie, CHU Hopital Michalon, Grenoble, France. *Dr R.L. Haberl*, Abteilung für Neurologie und Klinische Neurophysiologie, Städt.Krankenhaus München-Harlaching, München, Germany.

References

1. De Keyser J, Sulter G, Luiten PG. Clinical trials with neuroprotective drugs in acute ischaemic stroke: are we doing the right thing? *Trends Neurosci* 1999;22(12):535-40.

2. Gorelick PB. Neuroprotection in acute ischaemic stroke: a tale of for whom the bell tolls? *Lancet* 2000;355(9219):1925-6.

3. Lees KR. Neuroprotection. *Br Med Bull* 2000;56(2):401-12.

4. Lees KR. Cerestat and other NMDA antagonists in ischemic stroke. *Neurology* 1997;49:S66-S69.

5. Buchan AM, Lesiuk H, Barnes KA et al. AMPA antagonists: do they hold more promise for clinical stroke trials than NMDA antagonists? *Stroke* 1993;24:I148-I152.

6. Turski L, Huth A, Sheardown M et al. ZK200775: a phosphonate quinoxalinedione AMPA antagonist for neuroprotection in stroke and trauma. *Proc Natl Acad Sci U S A* 1998;95(18):10960-5.

7. Hardemark HG, Ericsson N, Kotwica Z et al. S-100 protein and neuron-specific enolase in CSF after experimental traumatic or focal ischemic brain damage. *J Neurosurg* 1989;71(5 Pt 1):727-31.

8. Persson L, Hardemark HG, Gustafsson J et al. S-100 protein and neuron-specific enolase in cerebrospinal fluid and serum: markers of cell damage in human central nervous system. *Stroke* 1987;18(5):911-8.

9. Herrmann M, Vos P, Wunderlich MT, de Bruijn CH, Lamers KJ. Release of glial tissue-specific proteins after acute stroke : A comparative analysis of serum concentrations of protein S-100B and glial fibrillary acidic protein. *Stroke* 2000;31(11):2670-7.

10. Missler U, Wiesmann M, Friedrich C, Kaps M. S-100 protein and neuron-specific enolase concentrations in blood as indicators of infarction volume and prognosis in acute ischemic stroke. *Stroke* 1997;28(10):1956-60.

11. Wunderlich MT, Ebert AD, Kratz T, Goertler M, Jost S, Herrmann M. Early neurobehavioral outcome after stroke is related to release of neurobiochemical markers of brain damage. *Stroke* 1999;30(6):1190-5.

12. Elting JW, de Jager AEJ, Teelken AW et al. Comparison of serum S-100 protein following stroke and traumatic brain injury. *J Neurol Sci* 2000;181(1-2):104-10.

13. Fassbender K, Schmidt R, Schreiner A et al. Leakage of brain-originated proteins in peripheral blood: temporal profile and diagnostic value in early ischemic stroke. *J Neurol Sci* 1997;148(1):101-5.

14. Buttner T, Weyers S, Postert T, Sprengelmeyer R, Kuhn W. S-100 protein: serum marker of focal brain damage after ischemic territorial MCA infarction. *Stroke* 1997;28(10):1961-5.

15. Gao F, Harris DN, Sapsed-Byrne S, Sharp S. Neurone-specific enolase and Sangtec 100 assays during cardiac surgery: Part III--Dose haemolysis affect their accuracy? *Perfusion* 1997;12(3):171-7.

16. Johnsson P. Markers of cerebral ischemia after cardiac surgery. *J Cardiothorac Vasc Anesth* 1996;10(1):120-6.

17. Wityk RJ, Pessin MS, Kaplan RF, Caplan LR. Serial assessment of acute stroke using the NIH Stroke Scale. *Stroke* 1994;25(2):362-5.

18. Matthews JNS, Douglas G, Canpbell MJ, Royston P. Analysis of serial measurements in medical research. *BMJ* 1990;300:230-5.

19. Dunnett CW. Pairwise multiple comparisons in the unequal variance case. *J Amer Statist Assoc* 1980;75:796-800.

20. Kim JS, Yoon SS, Kim YH, Ryu JS. Serial measurement of interleukin-6, transforming growth factor-beta, and S-100 protein in patients with acute stroke. *Stroke* 1996;27(9):1553-7.

21. Sulter G, Elting JW, De Keyser J. Increased serum neuron specific enolase concentrations in patients with hyperglycemic cortical ischemic stroke. *Neurosci Lett* 1998;253(1):71-3.

22. Browne SE, McCulloch J. AMPA receptor antagonists and local cerebral glucose utilization in the rat. *Brain Res* 1994;641(1):10-20.

23. Suzdak PD, Sheardown MJ. Effect of the non-NMDA receptor antagonist, 2,3-dihydro-6-nitro-7-sulfamoylbenzo(f)quinoxaline, on local cerebral glucose uptake in the limbic forebrain. *J Neurochem* 1993;61(4):1577-80.

24. Tacconi MT. Neuronal death: is there a role for astrocytes? *Neurochem Res* 1998;23(5):759-65.

25. Fillenz M, Lowry JP, Boutelle MG, Fray AE. The role of astrocytes and noradrenaline in neuronal glucose metabolism. *Acta Physiol Scand* 1999;167(4):275-84.

26. Barnes JM, Dev KK, Henley JM. Cyclothiazide unmasks AMPA-evoked stimulation of [3H]-L-glutamate release from rat hippocampal synaptosomes. *Br J Pharmacol* 1994;113(2):339-41.

27. Patel DR, Croucher MJ. Evidence for a role of presynaptic AMPA receptors in the control of neuronal glutamate release in the rat forebrain. *Eur J Pharmacol* 1997;332(2):143-51.

28. Gaspary HL, Simon RP, Graham SH. BW1003C87 and NBQX but not CGS19755 reduce glutamate release and cerebral ischemic necrosis. *Eur J Pharmacol* 1994;262(3):197-203.

29. Reichelt W, Stabel-Burow J, Pannicke T, Weichert H, Heinemann U. The glutathione level of retinal Muller glial cells is dependent on the high-affinity sodium-dependent uptake of glutamate. *Neuroscience* 1997;77(4):1213-24.

30. Kato S, Ishita S, Sugawara K, Mawatari K. Cystine/glutamate antiporter expression in retinal Muller glial cells: implications for DL-alpha-aminoadipate toxicity. *Neuroscience* 1993;57(2):473-82.

31. Khurgel M, Koo AC, Ivy GO. Selective ablation of astrocytes by intracerebral injections of alpha-aminoadipate. *Glia* 1996;16(4):351-8.

32. McBean GJ. Inhibition of the glutamate transporter and glial enzymes in rat striatum by the gliotoxin, alpha aminoadipate. *Br J Pharmacol* 1994;113(2):536-40.

33. Davis SM, Lees KR, Albers GW et al. Selfotel in acute ischemic stroke : possible neurotoxic effects of an NMDA antagonist. *Stroke* 2000;31(2):347-54.